EXCIPIENTS—SAFETY TESTING

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INTRODUCTION

The safety issues concerning pharmaceutical excipients can be classified into three categories: quality, toxicology, and improper use (1). Various regulatory directives address the quality category. In addition, the International Pharmaceutical Excipient Council's (IPEC) guideline publications address this issue by following the Organization of International Standardization (ISO) 9000 structure. IPEC is an industry association with worldwide membership that includes over 200 pharmaceutical, chemical, and food processing firms that develop, manufacture, sell, and use pharmaceutical excipients. IPEC comprises three regional organizations located in the United States, Europe, and Japan each with the same objectives.

Quality

The aforementioned IPEC guidelines that address quality include:

- Good Manufacturing Guide for Bulk Pharmaceutical Excipients
- Good Manufacturing Practices (GMP) Audit Guideline for Distributors of Bulk Pharmaceutical Excipients
- IPEC-Americas Significant Change Guide for Bulk Pharmaceutical Excipients
- GMP Audit Guideline for Suppliers of Bulk Pharmaceutical Excipients
- New Excipient Safety Evaluation Guidance
- IPEC-Americas Guide for the Development of an Impurity Profile
- Format and Required Content of Certificates of Analyses

The IPEC-Americas Safety Guidelines (modified) are presented as an information chapter in United States Pharmacopoeia (USP) 24/NF 19 (2). The Good Manufacturing Guide or Practices for Bulk Pharmaceutical Excipients also has been published as an information chapter in USP 24/NF 19. The guideline also serves as a basis for the World Health Organization (WHO) guidance to its national members (3). The intention of IPEC is to ensure that these guidelines reflect the concerns and intentions of responsible parties in the United States, the European Union (EU), and Japan. In other words, the guidelines are harmonized so that excipients that meet the requirements of a harmonized monograph can be sold and used in these three areas of the world. The use of these and other national guidelines ensure the quality of excipients.

These guidelines, using an ISO 9000 format, not only provide a way to assess whether systems are in place, but provide a means for evaluating the effectiveness of the systems. They also provide guidance on how to conduct an audit of a manufacturing operation that produces excipients (4) and in turn, give guidelines on auditing their distribution and repackaging (5).

International Conference on Harmonization Residual Solvent Guidance

Excipient impurity profiles and how to evaluate this important aspect of excipient manufacture, particularly in light of the International Conference on Harmonization (ICH) guidance published in 1999 (6), also are addressed. Care must also be taken that residual solvent levels do not exceed those proscribed in the ICH Guidance for Residual Solvents published in 1999. Solvents are divided into three classes:

1. *Class 1 solvents: Solvents to be avoided*. These include known human carcinogens, strongly suspected human carcinogens, and environmental hazards.
2. *Class 2 solvents: Solvents to be limited*. These include nongenotoxic animal carcinogens or possible causative agents of other irreversible toxicity, such as neurotoxicity or teratogenicity and solvents suspected of other significant but reversible toxicities.

3. *Class 3 solvents: Solvents with low toxic potential*. These include solvents with low toxic potential to man; no health-based exposure limit is needed. Class 3 solvents have permitted daily exposures of 50 mg or more per day.

IPEC Significant Change Guidance

Two areas of concern to excipient makers and users have been those of significant change and certificates of analyses. Any change by the manufacturer of an excipient that alters an excipient's physical or chemical property from the norm or that is likely to alter the excipient's performance in the dosage form is considered significant (7). Regardless of whether there is a regulatory requirement to notify the local regulatory authority, the manufacturer has an obligation to notify its customers of significant change so that the customer can evaluate the change on the customer's products. The Significant Change Guidance establishes uniform considerations for evaluating significant changes involving the manufacture of bulk excipients. The types of changes that might be considered include:

Site
Scale
Equipment
Process
Packaging
Specifications

The requirement for evaluating the impact of change on the excipient begins at the processing step from which GMP compliance begins, as noted in the IPEC Good Manufacturing Guide or Practices Guide for Bulk Pharmaceutical Excipients, or later in the process.

The evaluation criteria in the guideline include:

1. Changes in the chemical properties of the excipient owing to the change
2. Changes in the physical properties of the excipient owing to the change
3. Changes in the impurity profile of the excipient owing to the change
4. Changes in the functionality of the excipient owing to the change
5. Changes in the moisture level of the excipient owing to the change
6. Changes in the bioburden of the excipient owing to the change

The guideline also provides for consideration of objective criteria when considering changes to the impurity profile of an excipient as a result of any change. IPEC-Americas has developed a guide for the preparation of an impurity profile for excipients. The profile addresses the following:

1. All specified organic impurities
2. Unidentified organic impurities at or above 0.1% whether specified or not, unless the impurity has an established pharmacological effect or is known to be unsafe at a lower level
3. Residual solvents
4. Inorganic impurities
5. Toxic impurities

The content of the impurity profile varies with the nature of the excipient, the raw materials used in its manufacture, and its chemical composition. Changes are considered significant whenever a new impurity is introduced at or above the 0.1% concentration or when an impurity previously present at or above 0.1% disappears.

IPEC Certificate of Analysis Guidance

The second issue involves the certificate of analysis that the manufacturer must provide to the formulator when shipping the excipient. Most often, a certificate of analysis does not contain information developed as a result of analysis of the specific batch of material being delivered. The analysis may have either been conducted on previous individual batches or on a mixture of aliquots of previous batches. No guidelines regarding exactly what should be found in the certificate and how it should be presented have been established. This is addressed in the guideline (8). At the time of this writing, the frequency of sampling has not been resolved with the U.S. Food and Drug Administration (FDA). Some believe that in the face of no significant changes it should not be necessary to sample each manufactured batch, but that there is a need only for sufficient sampling to ensure that statistical significance of sampling results can be met. What to do if the manufacturing process is continuous rather than a batch process would fall under the same criteria except that the sampling frequency would probably be based on time/volume rather than batches.

EXCIPIENT USAGE

There are roughly 8000 "nonactive" ingredients being used in food, cosmetics, and pharmaceuticals worldwide (1). In 1996, approximately 800 excipients were used in

marketed pharmaceutical products in the United States (1). Although the FDA maintains a "list" of inactive ingredients, the EU and other European countries do not have official published lists, although steps are being taken to rectify this situation.

Few excipients are manufactured specifically for pharmaceutical use. Many are manufactured for other purposes (e.g., food, cosmetics, paint thickeners, construction, etc.). For their use in pharmaceuticals, additional quality, functionality, and safety requirements must be met.

Improper Use of Excipients

The improper use of excipients is addressed, to a certain extent, by the package inserts found in the formulated products. The challenge is to educate consumers and health providers to read and comply with the information contained in these inserts.

DEFINITION OF AN EXCIPIENT

For toxicological purposes, it may be inappropriate to define excipients as inert ingredients. It may be more appropriate to define an excipient (9) as "Any substance other than the active drug or pro-drug which has been appropriately evaluated for safety and is included in a drug delivery system to either:

1. Aid processing of the system during manufacture
2. Protect, support, or enhance stability, bioavailability
3. Assist in product identification
4. Enhance any other attribute of the overall safety and effectiveness of the drug product during storage and use."

As the fourth definition indicates, excipients include a multiplicity of activities from mold releasers to absorption enhancers, and more recently include substances that permit large molecule (e.g., proteins) to be absorbed from the gastrointestinal tract without degradation. Most actions by an excipient are mechanical rather than pharmacological.

APPROVAL MECHANISMS FOR EXCIPIENTS

Currently, regulatory agencies have not established safety-testing guidelines specifically for excipients (10–13). Under U.S. law, a new pharmaceutical excipient, unlike an active drug, has no regulatory status unless it can be qualified through one or more of the approval mechanisms available for components used in finished drug dosage forms. These approval mechanics include:

- Generally Recognized As Safe (GRAS) determination pursuant to 21 Code of Federal Regulations (CFR) 182, 184, and 186
- Approval of a food additive petition under 21 CFR 171
- As contained in a New Drug Application (NDA) approval for a specific drug product and for a particular function or use in that dosage form

Within the EU there is a directive that makes it clear that new chemical excipients will be treated in the same way as new actives (14).

TOXICITY TESTING

The very nature of excipients, for the most part, represents unique problems in testing for toxicity. The actions sought for many excipients are mechanical rather than physiological. Exceptions to this are flavors (12). A most desirable description of some excipients would include being pharmacologically inert and mechanically functional. An alternative would be one where the toxic dose was so high as to be meaningless while still retaining functionality requirements through a range of high and low doses. The acceptable risk for a traditional excipient, when compared to an active principle in a formulation, is generally several orders of magnitude different. Unless an excipient has some very unique properties, it is unlikely that a new excipient would be developed that did not have a large safety factor for toxicity and side effects under conditions of use.

As excipients become more complex and are required to perform functions not required in the past, it is conceivable that a distinction will have to be made between excipients and what might be termed "co-drugs." The use of monoclonal antibodies to deliver an active principle to a specific tissue site might be considered an example of this diversity.

In 1994, as part of the IPEC-Americas program to obtain stand-alone status for excipients, a safety committee was formed. The committee was composed of men and women from a variety of medical and chemistry disciplines who were directed to develop safety-testing guidelines for new excipients. These guidelines were published in 1996 (12). At that time, regulations in most developed countries did not address registration of an excipient as a separate entity. For example, the drug

master file for an excipient in the United States is reviewed only as part of the NDA process. Inherent to the current process is the assumption that the use of an excipient in an approved drug dosage form ensures its acceptance in other dosage forms and its ultimate inclusion in the National Formulary (NF). The NF monographs provide standards/specifications for identity, purity, and analysis. Priority for inclusion is given to formulations with approved NDAs and those approved for use in foods. The FDA favors the use of commercially established excipients, such as food additives and substances that have been designated GRAS.

The guidelines developed by IPEC-Americas (12) provide for a tier approach to required testing. The tests to be conducted are based upon the route of application of the formulated drug and the duration of use. A base set of data is required for all candidate excipients. The guidelines require a review of the chemical and physical properties of the excipient and a review of the scientific literature, exposure conditions (including dose, duration, frequency, route, and user population), and absence or presence of pharmacological activity.

Alternatives to the use of living animals are encouraged wherever these procedures have been validated. The information will provide sufficient information upon which to base a safety judgment and the data will be acceptable to a regulatory agency. The studies should also follow the appropriate legal and professional codes (15) in the conduct of all tests and should meet the Good

Table 1 Summary IPEC-America safety testing guidelines

	Routes of exposure for humans:					
Tests	**Oral**	**Mucosal transdermal/ injectable topical**			**Inhalation/ intranasal**	**Ocular**
Baseline toxicity data						
Acute oral toxicity	R	R	R	R	R	R
Acute dermal tox.	R	R	R	R	R	R
Acute inhalation tox.	C	C	C	C	R	C
Eye irritation	R	R	R	R	R	R
Skin irritation	R	R	R	R	R	R
Skin sensitization	R	R	R	R	R	R
Acute parenteral tox.	—	—	—	R	—	—
Application site eval.	—	R	R	R	R	—
Pulmonary sensitization	—	—	—	—	R	—
Phototoxicity/allergy	—	—	R	—	—	—
Bacterial gene mutation	R	R	R	R	R	R
Chromosomal damage	R	R	R	R	R	R
ADME—intended route	R	R	R	R	R	R
28-day toxicity (2 species) intended route	R	R	R	R	R	R
Additional data: Short- or intermediate-term repeated use						
90-day toxicity (most appropriate species)	R	R	R	R	R	R
Embryo-fetal toxicity	R	R	R	R	R	R
Additional assays[a]	C	C	C	C	C	C
Genotoxicity	R	R	R	R	R	R
Immunosupression (3)	R	C	C	R	R	R
Additional data: Intermittent long-term or chronic use						
Chronic toxicity (rodent Nonrodent)	C	C	C	C	C	C
1-generation reproduction	R	R	R	R	R	R
Photocarcinogenicity	—	—	C	—	—	—
Carcinogenicity	C	C	C	C	C	C

Note: R, required; C, conditional

[a]Additional assays are dependent on the judgment of the data evaluator. They may include, but are not limited to screening for endocrine modulators or tests to determine if findings in animals are relevant to humans.

(From Ref. 12, p. 53.)

Laboratory Practices of the agency-(ies) to which the data will be submitted.

The base set of data is designed to provide fundamental information regarding acute toxicity by the oral route and/or intended dose route (Table 1). Skin and eye irritation testing should be conducted irrespective of the route of use of the candidate excipient. These data are intended to protect researchers during the research and production life of the material.

Absorption/distribution/metabolism/excretion/pharmacodynamics are considered fundamental data, as are mutagenicity tests (e.g., Ames test, in vivo chromosome aberration test, and mouse micronucleus test). Twenty-eight day repeated dosing studies in two species by appropriate route(s) also should be performed in a rodent and a nonrodent species, respectively.

One of the unique aspects of the IPEC approach is that not all tests are required. Some of the tests are conditional upon findings in other test procedures. Specific attention is paid to the route of exposure as well as to tests that might be required as potential exposure duration is increased. Emphasis is placed on the fact that the route of exposure for the test animals should be the same as the route of exposure anticipated in humans. Strict attention is paid to the type of exposure. For example, a protocol for study of a product intended for inhalation therapy that results in prolonged exposure of up to several hours per day will differ from that used to evaluate a material that would be used in a product resulting in exposure to several metered doses each day. Some tests may have to be conducted using a route of exposure different from the intended use route. This may be due to the nature of the test animal (e.g., reproductive tests in rabbits may require that the dosage route be other than inhalation, if inhalation is to be the route of use of the formulation containing the excipient).

The IPEC-Americas publication emphasizes that untrained people should not use its guidelines. In addition, the guidelines are not to be used as a checklist. They are to be used by professionals qualified to make the necessary judgments concerning what is referred to as "Conditional" tests. The conduct of these conditional tests is dependent on the results obtained from other required tests. It was considered that given the specificity of some of the cellular and subcellular techniques available and the variety of test animals being developed, that the traditional long-term imprecise test procedures may produce irrelevant information compared to that available from other test procedures. Also, some chemical families produce false positive or questionable results in certain species and the development of these types of data only serve to confound and require additional testing to clarify the questionable results.

It is conceivable that some excipients may not require the standard 2-year, two rodent species carcinogenicity studies. Such excipients include those that are not absorbed (or are rapidly metabolized and/or rapidly excreted), that do not exhibit toxicity in 90-day studies, and those that are negative for genotoxicity. This is the approach taken by the IPEC-Americas Safety Committee and one of the reasons that the 1996 peer-reviewed journal publication (12) indicates that the conduct of rodent carcinogenicity studies is conditional. The carcinogenicity studies that are conditional are the traditional 50 animals/sex/group rodent studies conducted for 18 or 24 months or variations thereof. The decision to make these tests conditional was also predicated on the fact that other models, that provided adequate information upon which to base a safety judgment regarding carcinogenic potential, were available.

GENETICALLY ENGINEERED ANIMAL MODELS

The use of genetically engineered animals has the potential to supplant some of the traditional long-term (2-year) rodent studies. Mouse models have been developed for use as mechanistic models in cancer research. Potential alternatives to the 2-year rodent oncogenicity bioassay include the p53 knockout mouse and the Tg.AC mouse (16).

The use of these mouse models is based on the observation that human neoplasms commonly demonstrate molecular alterations in tumor suppressor genes and/or oncogenes. In normal tissues, tumor suppressor genes (such as p53 and Rb) serve as negative regulators of cell proliferation. Inactivation or loss of tumor suppressor activity through gene mutation or deletion results in loss of this critical regulatory function and may lead to uncontrolled cell proliferation.

Loss of tumor suppressor gene is the most common genetic alteration found in human cancers. Deletion of one or both alleles of p53 (p53 knockout mice) increases the incidence of neoplasia and decreases latency of tumor development. When p53 knockout mice are exposed to genotoxic agents, they rapidly develop neoplasms in a range of tissues. Sensitive targets in p53 mice are often comparable to those in "normal" mice and hence, their utility as a model.

The Tg.AC mouse is used as a skin tumorigenesis model, and when exposed to phorbol ester tumor promoters and other nongenotoxic agents, is rapidly induced. When fully validated, a test battery, including the

Table 2 Summary of IPEC-Europe excipient testing guidelines

	Routes of exposure for humans:						
Tests	**Oral**	**Mucosal**	**Transdermal**	**Dermal/topical**	**Parenteral**	**Inhalation/intranasal**	**Ocular**
Step 0							
ADME	R	R	R	R	R	R	R
Step 1 (Basic set)							
Acute oral toxicity (intended route)	R	R	R	R	R	R	R
Eye irritation	—	R	R	R	R	R	R
Skin irritation	—	R	R	R	R	R	R
Skin sensitization	R	R	R	R	R	R	R
Acute parenteral toxicity	—	—	—	—	R	—	—
Application site evaluation	—	R	R	R	R	—	—
Pulmonary sensitization	—	—	—	—	—	C	—
Phototoxicity/photo- allergly	—	—	C	C	—	—	—
Ames test	R	R	R	R	R	R	R
Chromosome damage	R	R	R	R	R	R	R
Micronucleus	R	R	R	R	R	R	R
4 weeks toxicity 2(species)—intended route	R	R	R	R	R	R	R
Step 2							
90-day toxicity (most appropriate species)	R	R	R	R	R	R	R
Teratology (rat and rabbit)	R	R	R	R	R	R	R
Genotoxicity assays	R	R	R	R	R	R	R
Step 3							
6—9 months chronic toxicity (Rodent, nonrodent)	C	C	C	C	C	C	C
Segment 1	R	R	R	R	R	R	R
Segment III	C	C	C	C	C	C	C
Photocarcinogenicity	—	—	C	C	—	—	—
Carcinogenicity	C	C	C	C	C	C	C

Note: R, required C, conditional.
(From the IPEC Europe Safety Committee. The Proposed Guidelines for the Safety Evaluation of New Excipients. European Pharmaceutical Review, Nov 1997.)

heterozygous p53 knockout mouse and the Tg.AC mouse, may provide a model which will identify both genotoxic and nongenotoxic carcinogens and reduce the in-life time to conduct studies for carcinogenicity to as little as 6 months.

SUMMARY

The tests suggested by IPEC-Americas are summarized in Table 1 (12). The "R" represents required tests and the "C" represents tests that are conditional based on intended use and the results of previous tests. The tests suggested by IPEC-Europe (18) are found in Table 2 and differ slightly from Table 1. The decision whether or not to perform "C" labeled tests requires the judgment of a trained professional. Both IPEC test models are also predicated on obtaining chemical, pharmacological, and physical data from other investigators involved in the development of candidate excipients. Information developed by chemists, pharmacologists, and other disciplines is invaluable in estimating the hazards associated with a new compound.

Testing in humans, using the IPEC-Americas model, either as part of a clinical trial or as a stand-alone procedure, should be conducted as soon as warranted by the animal data. Critical evaluation of the base- set data may support the use of a candidate excipient intended for use once or twice in a lifetime. If one conducts the studies listed in Table 1, section 2, critical evaluation of the data may support the use of the new excipient in a variety of products intended for limited repeated intake, for example an antibiotic. If the Absorption, Distribution, Metabolism, Excretion/Pharmacokinetics (ADME/PK) studies show that the excipient is not absorbed, review of the other data may permit inclusion in a product intended to be used for 30–90 consecutive days. For longer-term usage, the tests listed in Table 1, section 3 must be considered. One-generation reproduction studies must be conducted to assess any excipient-induced effects/disturbances in mating behavior, development/maturation of gametes, fertility, and preimplantation/implantation loss of embryos. Should the data continue to support some concern for either reproductive or developmental toxicity, a segment III study might be appropriate (3, 19, 20).

Specific details regarding test methodology are not provided in the guideline. Test procedures generally recognized by experts and the regulatory agencies should be used. Each test should be designed to address a specific issue and the data should be evaluated accordingly. Care should be taken when evaluating animal data to ensure that toxicological findings are not unique to the particular test species and therefore not relevant to the human experience.

Finally, it is important that a material being evaluated for safety is the same as that which will be used in pharmaceutical preparations. The manufacturer of the excipient must follow GMPs. A complete audit trail must be available from the time of manufacture until the product is made available to the consumer. IPEC-Americas has developed a third-party audit program that follows the guidelines enumerated above. The program is conducted by the International Pharmaceutical Excipients Audits, Inc. (IPEA) and is the only program of its type that focuses only on the quality of pharmaceutical excipients.The program is designed to prevent problems with excipients, such as the one that occurred in Haiti in 1996, where 80 children died because the glycerol in their cough medication was mostly glycol.

Ultimately, the safety of an excipient in a formulation requires the following:

1. That the excipient being used is the same excipient that was tested for safety.
2. The test procedures were adequate to evaluate safety and are acceptable to relevant regulatory authorities.
3. The excipient is as specified.
4. The formulated pharmaceutical is used as specified.
5. The concentration of the excipient in the formulation takes into account appropriate test data.

REFERENCES

1. De Jong, H.J. The Safety of Pharmaceutical Excipients. *Therapie* **1999**, *54* (11).
2. The United States *Pharmacopoeia 24/National Formulary 19; 2000*.
3. Principles for the Safety Assessment of Food Additives and Contaminants in Food. World Health Organization. Environ. Health Criter. **1987**, *70* .
4. *Good Manufacturing Practices Guide for Bulk Pharmaceutical Excipients*; The International Pharmaceutical Excipients Council: Arlington, VA, 2000.
5. *The International Pharmaceutical Excipients Council Guideline for Distribution of Bulk Pharmaceutical Excipients; 2000*.
6. Guideline for Residual Solvents, *International Conference on Harmonization; 1999*.
7. IPEC-Americas Significant Change Guide for Bulk Pharmaceutical Excipients, The International Pharmaceutical Excipients Council of the Americas, 2000.
8. Guidance on the Required Content and Format of Certificates of Analysis, The International Pharmaceutical Excipients Council of the Americas, 2000.

9. Blecher, L. Excipients: The Important Components. Pharm. Process **1993**.
10. Cooper, J. Inert Components of Pharmaceutical Preparations. Drug Dev. Ind. Pharm. **1979**, *5*, 293.
11. Smith, J.M.; Dodd, T.R.P. Adverse Drug Reactions. Acute Poisoning Review **1982**, *1*, 93.
12. Steinberg, M.; Borzelleca, J.F.; Enters, E.K.; Kinoshita, F.K.; Loper, A.; Mitchell, D.B.; Tamulinas, C.B.; Weiner, M.L. A New Approach to the Safety Assessment of Pharmaceutical Excipients. Regul. Toxicol. Pharmacol. **1996**, *24* (2), 149.
13. Steinberg, M. ICH Guidelines and Safety: An Update. Pharm. Tech. **1999**.
14. E.U. Directive 75/318 European Economic Community, 1995.
15. Guiding Principles in the Use of Animals in Toxicology Society of Toxicology, 1996.
16. *Life Sciences News Letter*; ITT Research Institute: Chicago IL, 2000.
17. Moreton, C.R. New Excipients: From Idea to Market. Eur. Pharma. Rev. **1997**, *2* (3).
18. The IPEC Europe Safety Committee. The Proposed Guidelines for the Safety of New Excipients. Eur. Pharm. Rev. **1997**, November.
19. Bass, R.; Ulbrich, B.; Hildebrandt, A.G.; Weissinger, J.; Doi, O.; Baeder, C.; Fumero, S.; Harada, Y.; Lehmann, H.; Manson, J.; Neubert, D.; Omori, Y.; Palmer, A.; Sullivan, F.; Takayama, S.; Tanimura, T. Draft Guideline on Detection of Toxicity of Reproduction for Medicinal Products. Adv. Drug React. Toxicol. Rev. **1991**, *9* (J), 127.
20. Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Foods, Redbook II: Draft, *U.S. Food and Drug Administration (USFDA), Bureau of Foods; 1993*.
21. Weiner, M.L.; Kotloskie, L.A. *Excipient Toxicity and Safety*; Marcel Dekker, Inc.: New York, 1999.